Product Manual

OxiSelect™ s-Glutathione Adduct Competitive ELISA Kit

Catalog Number

STA-814 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Oxidative stress occurs when there is an excess of free radicals such as reactive oxygen species (ROS) or reactive nitrogen species (RNS) in the body. Research has shown that excessive ROS/RNS accumulation will lead to cellular injury, such as damage to DNA, proteins, and lipid membranes. ROS/RNS damage has been implicated in the development of many physiological problems, such as ageing, asthma, arthritis, diabetes, cancer, inflammation, cardiovascular disease, atherosclerosis, Down's syndrome, and neurodegenerative diseases.

Upregulation of ROS/RNS results in the oxidative modification of proteins. ROS can modify the sulfhydryl group on a protein to form either sulfonic, sulfenic, or sulfinic acid derivatives. These derivatives can form intramolecular disulfide bonds, or react with other sulfhydryl containing biomolecules. Glutathione contains such a sulfhydryl moiety, composed of glutamic acid, cysteine, and glycine, and can be found in abundance in cells where it serves to maintain redox balance. While glutathione helps protect cells from free radical damage by acting as an antioxidant, glutathione can also covalently attach to proteins through the derivatized sulfhydryl moiety serving as a marker of oxidative stress. Levels of s-glutathionylated proteins in human serum have recently been demonstrated to be a sensitive risk-marker for arteriosclerosis obliterans.

When a cysteine sulfhydryl moiety is s-glutathionylated, the resulting modification can affect the activity of the modified protein. Some proteins such as peroxiredoxin 6 and sarco/endoplasmic reticulum calcium (Ca(2+)) ATPase (SERCA) are activated upon s-Glutathionylation, while others such as beta-actin, endothelial nitric oxide synthase (eNOS), annexin A2, tubulin, the ATP-dependent Na^+/K^+ pump, and protein kinase C are downregulated. Therefore, s-Glutathionylation is both a marker for oxidative stress and a regulator of protein function.

The OxiSelect™ s-Glutathione Adduct Competitive ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of s-Glutathione-protein adducts. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples.

Assay Principle

First, an s-glutathione conjugate is coated on an ELISA plate. The unknown s-glutathionylated protein samples or s-glutathione-BSA standards are then added to the s-glutathione conjugate preabsorbed ELISA plate. After a brief incubation, an Anti-s-Glutathione Adduct-Specific Monoclonal Antibody is added, followed by an HRP conjugated Secondary Antibody. The relative amount of s-glutathione protein adducts in unknown samples is determined by comparison with a predetermined s-glutathione-BSA standard curve.

Related Products

1. STA-300: OxiSelect™ N^{ε}-(carboxyethyl) lysine (CEL) ELISA Kit
2. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
3. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
4. STA-311: OxiSelect™ Methylglyoxal (MG) ELISA Kit
5. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)

6. STA-816: OxiSelect™ N^{ε}-(carboxymethyl) lysine (CML) Competitive ELISA Kit
7. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit
8. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit
9. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-s-Glutathione Adduct Antibody (500X) (Part No. 281401): One 10 µL vial of Anti-s-Glutathione Adduct Antibody.
3. Secondary Antibody, HRP Conjugate (Part No. 230003): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. s-Glutathione-BSA Standard (Part No. 281402): One 50 µL vial of 1 mg/mL s-Glutathione-BSA in PBS.
2. s-Glutathione Conjugate (Part No. 281403): One 20 µL vial of 0.2 mg/mL s-Glutathione Conjugate in PBS.
3. 100X Conjugate Diluent (Part No. 281603): One 300 µL vial.

Materials Not Supplied

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. 1X PBS
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Anti-s-Glutathione Adduct Antibody, s-Glutathione-BSA Standard, s-Glutathione Conjugate and 100X Conjugate Diluent at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

Preparation of Reagents

- s-Glutathione Conjugate Coated Plate:

  Note: The s-Glutathione Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

  1. Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 100 µL to 9.9 mL of 1X PBS. Excess 1X Conjugate Diluent can be stored at 4ºC for future use.
  2. Immediately before use, prepare 100 ng/mL of s-Glutathione Conjugate by diluting the 0.2 mg/mL s-Glutathione Conjugate in 1X Conjugate Diluent. Example: Add 5 µL of 0.2 mg/mL s-Glutathione Conjugate to 95 µL of 1X Conjugate Diluent to make 10 µg/mL s-Glutathione Conjugate and then add 50 µL of 10 ug/mL s-Glutathione Conjugate to 4.950 mL of 1X Conjugate Diluent and mix well.
  3. Add 100 µL of the **100 ng/mL** s-Glutathione Conjugate to each well and **incubate overnight at 4ºC**. Remove the s-Glutathione Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4ºC until ready to begin the assay.

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-s-Glutathione Adduct Antibody and Secondary Antibody: Immediately before use, dilute the Anti-s-Glutathione Adduct Antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of s-Glutathione-BSA standards in the concentration range of 0 to 10 µg/mL by diluting the s-Glutathione-BSA Standard in Assay Diluent (Table 1).

Standard Tubes	1 mg/mL s-Glutathione-BSA Standard (µL)	Assay Diluent (µL)	s-Glutathione-BSA (ng/mL)
1	5	495	10,000
2	100 of Tube #1	200	3333
3	100 of Tube #2	200	1111
4	100 of Tube #3	200	370
5	100 of Tube #4	200	123
6	100 of Tube #5	200	41
7	100 of Tube #6	200	14
8	0	200	0

Table 1. Preparation of s-Glutathione-BSA Standards

Assay Protocol

Note: If testing mouse or rat plasma or serum, the IgG must be completely removed from each sample prior to testing, such as with Protein A or G beads. Additionally, a control well without primary antibody should be run for each sample to determine background signal.

1. Prepare and mix all reagents thoroughly before use. Each sample including unknown and standard should be assayed in duplicate.
2. Remove the Assay Diluent from the s-Glutathione Conjugate Coated Plate and add 50 µL of unknown sample or s-Glutathione-BSA standard. If needed, unknown samples may be diluted in 1X PBS containing 0.1% BSA before adding. Incubate at room temperature for 5 minutes on an orbital shaker.
3. Add 50 µL of the diluted Anti-s-Glutathione Adduct Antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash 3 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 4 above.
6. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 2-20 minutes on an orbital shaker.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
7. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
8. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical s-Glutathione Adduct Competitive ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

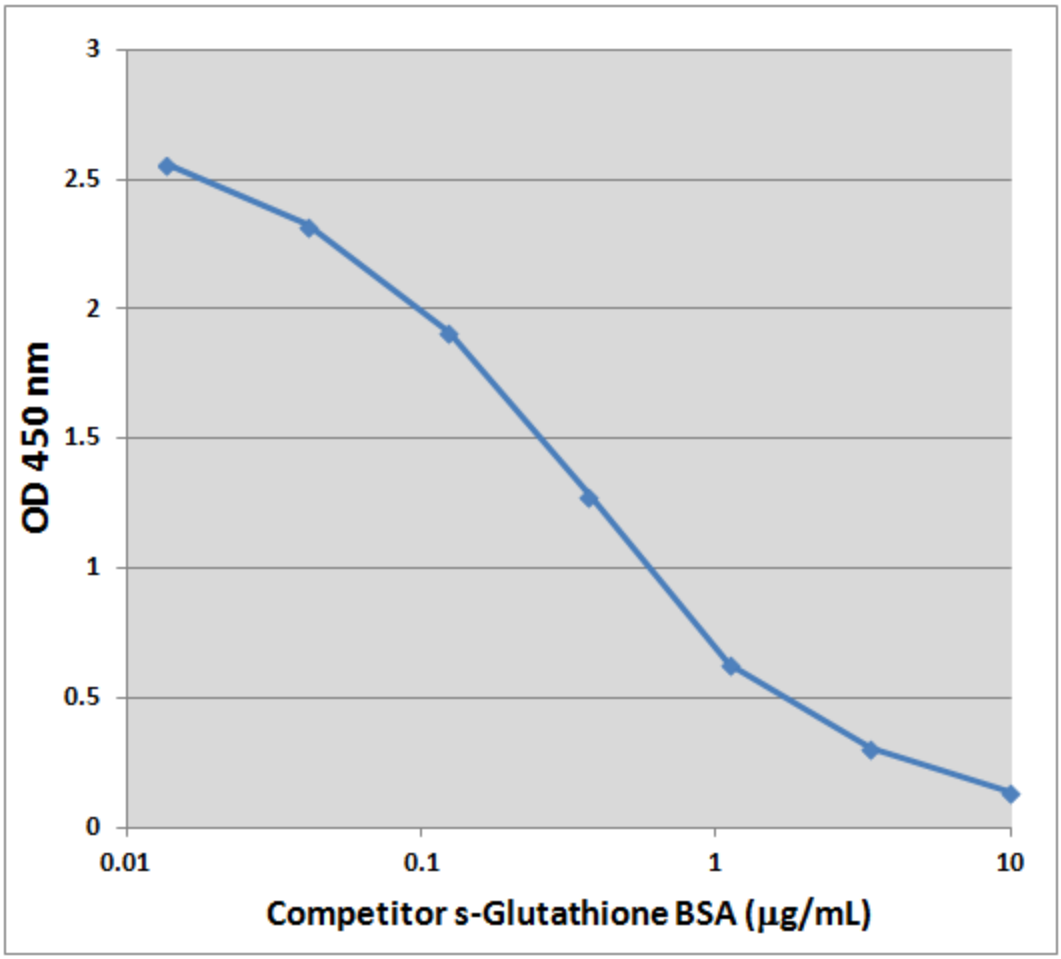

Figure 1: s-Glutathione-BSA Competitive ELISA Standard Curve.

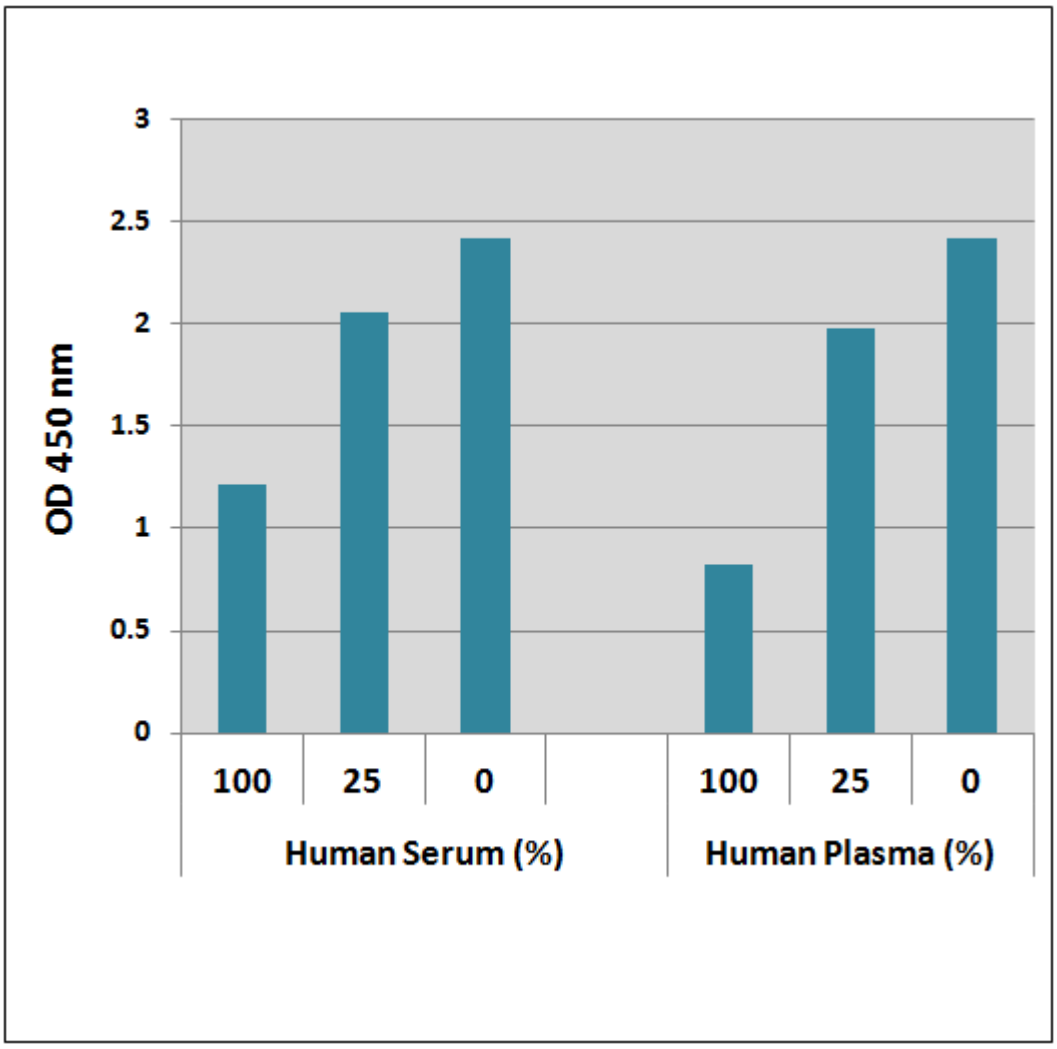

Figure 2: s-Glutathione Protein Adducts in Human Serum and Plasma. Competitor s-Glutathionylated protein levels were assayed in Human Serum (left) and Human Plasma (right).

References

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